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SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL (E)-N'-BENZYLIDENE-3-(PIPERAZIN-1-YL)BENZO[B]THIOPHENE-2-CARBOHYDRAZIDE DERIVATIVES

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ABSTRACT

A novel series of *(E)-N'*-benzylidene-3-(piperazin-1-yl)benzo[b]thiophene-2-carbohydrazide derivatives **(5a-5l)** were synthesised from the starting materials like cinnamic acid, thionyl chloride, chlorobenzene and pyridine resulting in synthesis of 3-Chlorobenzo(b)thiophene-2-carbonyl chlorides which were further reacted with hydrazine hydrate, aromatic aldehydes and finally with substituted piperazines to yield the titled compounds. Structural characterization of the synthesized compounds was carried out by instrumental method of analysis like IR, ^{1}H-NMR and Mass spectroscopy. The titled compounds were subjected to *in-vitro* antibacterial activity against bacteria *E. coli* and *S. aureus* and antifungal activity against fungi *C. albicans*. The biological results indicated that compound **5l** showed most potent antibacterial activity and **5c** displayed most potent antifungal activity on comparision with Ciprofloxacin and Fluconazole as respective standard drugs.

KEY WORDS: Benzothiophene, Piperazine, antibacterial, antifungal activity.

INTRODUCTION

Infectious diseases are amongst the most common diseases all over the world [1]. A recent World Health Organization report indicated that infectious diseases are responsible for more than 17 million deaths worldwide each year, most of which are associated with microbial

infections [2]. Hence, the control of infectious diseases is still an important task for medicinal chemists in the world. Deaths from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria and tuberculosis account for more than 85% of the mortality from infection worldwide [3].

Antimicrobial resistance is a global problem that needs urgent action. Microbial resistance has become a very serious clinical problem for many classes of antibiotics. Resistance to first-line drugs in the pathogens causing these diseases ranges from zero to almost 100%. The rise in antibiotic-resistant microorganisms in recent years has led to an increasing search for new antibiotics [4]. Therefore discovery and development of effective antimicrobial drugs with novel mechanism of action has become the highest priority task for researchers in this area [5].

Benzothiophene and Piperazine scaffolds are versatile heterocyclic nuclei by having a broad spectrum of pharmacological activities [6]. Benzothiophene substituted Piperazine derivatives in the literature have been found to exhibit superior antimicrobial activity as compared to compounds having individual benzothiophene or piperazine nucleus. These findings and problems discussed above have encouraged us for synthesis of substituted benzothiophene incorporated piperazine compounds and their antimicrobial evaluation for discovery of potent antimicrobial agents to solve menace of microbial resistance.

MATERIALS AND METHODS

TLC was performed on silica plates pre-coated with Merck Silica Gel 60 F254 by using UV lamp L179 or iodine vapors as visualizing agent to check the progress of chemical reactions as per the scheme of reactions. Melting points were determined with a Buchi 530 melting point apparatus in an open capillary tube and are uncorrected. Infrared spectra were recorded in KBr pellets on Bruker FTIR. The ^{1}HNMR spectra were recorded in DMSO-*d6* solution on a Bruker Avance 11 400 spectrophotometer operating at 400.00 MHz by using tetramethyl silane (TMS) as an internal reference (chemical shift in δ, ppm). The mass spectra were recorded on a Shimadzu 2010A LC-MS spectrometer. Chemicals and all solvents used in this experimental work were procured from Merck AG (Mumbai, India), SD Fines (Mumbai, India), Sigma Aldrich (Bangalore, India) and Qualigens (Navi Mumbai, India).

EXPERIMENTAL

The titled compounds were synthesized by following the scheme of reactions as shown in Figure 1 and general procedures about all steps involved in synthesis of benzothiophene substituted piperazine derivatives are presented as below.

General procedure for the synthesis of 3-Chlorobenzo[b]thiophene-2-carbonyl chloride, (2)

The synthesis of 3-Chlorobenzo[b]thiophene-2-carbonyl chloride **(2)** was carried out by addition to a stirred mixture of cinnamic acid **(1)** (74.1 g, 0.50 mol), pyridine (4.0 mL, 0.05 mol), thionyl chloride (150 mL, 0.77 mol) and chlorobenzene (300 mL) and heated at reflux for 48hr. Excess of thionyl chloride was removed under reduced pressure and the remaining material was suspended in hot n-hexane (800 mL) and then filtered. The hot filtrate was treated with charcoal, allowed to cool and then the crystalline yellow solid obtained, was filtered and recrystallized from ethanol.

General procedure for the synthesis of 3-chlorobenzo[b]thiophene-2-carbohydrazide, (3)

The synthesis of 3-chlorobenzo[b]thiophene-2-carbohydrazide **(3)** was carried out by dropwise addition of hydrazine hydrate (0.0018 mole) to a solution of compound **(2)** (0.001 mole) in dry chloroform (5 mL). The mixture was refluxed for one hour. The solvent was removed under vacuum and the solid product was collected and crystallized from ethanol.

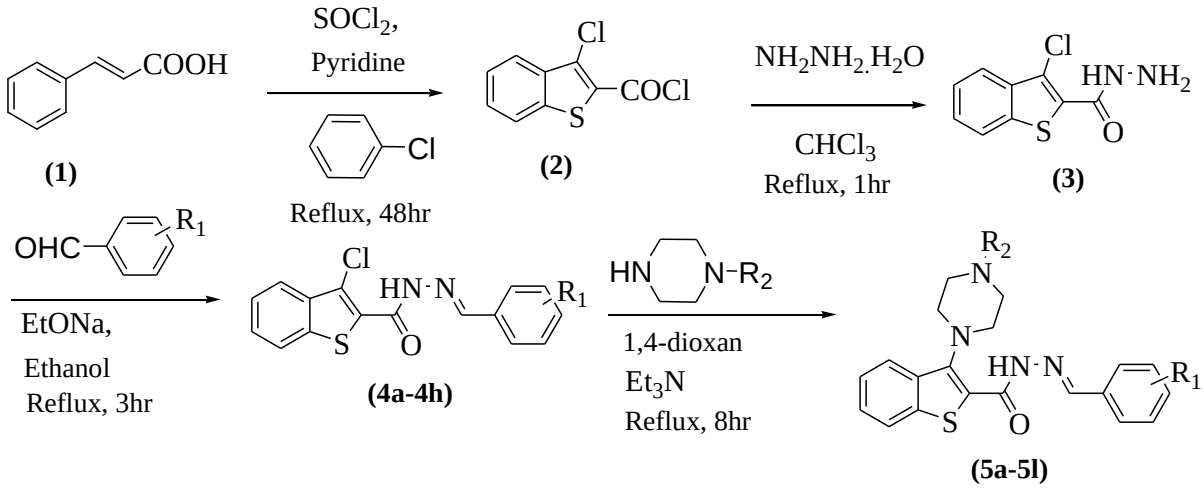
General procedure for the synthesis of N'-benzylidene-3-chlorobenzo[b]thiophene-2-carbohydrazide derivatives, (4a-4h)

The synthesis of *N'*-benzylidene-3-chlorobenzo[b]thiophene-2-carbohydrazide derivatives **(4a-4h)** was carried out by refluxing a mixture of **(3)** (2.2 g, 0.01 mol) and 1.06 g of substituted benzaldehyde in 20 ml of absolute ethanol and 2 g of sodium metal for 3 hours. The excess alcohol was distilled off under vacuum. The yellow solid separated was filtered and recrystalised from absolute ethanol.

General procedure for the synthesis of titled compounds (5a-5l)

The synthesis of *N'*-benzylidene-3-(piperazin-1-yl)benzo[b]thiophene-2-carbohydrazide derivatives **(5a-5l)** were carried out by addition of equimolar quantities of **(4a-4h)** (0.01 mol) and piperazine derivatives by stirring in 10 ml of 1,4-dioxan 2 ml of triethylamine for 10

minutes. The reaction mixture was then refluxed for 8 hours, cooled to room temperature. The solid separated was then filtered and recrystallised from absolute ethanol.



	R_1	R_2
5a;	3,4,5-OCH_3	H
5b;	4-$N(CH_3)_2$	H
5c;	CH_3-CH=CH-C_6H_4	H
5d;	2-OH	4-F -C_6H_4
5e;	2-OH,4-OCH_3	H
5f;	2-OH,4-OCH_3	4-F -C_6H_4
5g;	4-$N(CH_3)_2$	C_6H_5
5h;	4-Cl	H
5i;	4-Cl	C_6H_5
5j;	4-Br	H
5k;	4-Br	C_6H_5
5l;	4-NO_2	H

Figure 1: Synthesis of benzothiophene substituted piperazine derivatives

Evaluation of antimicrobial activities

Antimicrobial activities of the synthesized compounds against Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), Gram-negative bacteria *Escherichia coli* (ATCC 10798) and fungal stain *Candida albicans* (ATCC 10231) were expressed as zone of inhibition values in mm [7]. The zone of inhibition values were determined by cup plate agar diffusion method and measured in triplicate sets by using a scale. Zone of inhibition values of the synthesized compounds and the standard drugs, Ciprofloxacin and Fluconazole, were compared at concentration of 1000µg/ml [8]. The stock solutions of the compounds were prepared in dimethyl sulphoxide (DMSO) as solvent which was also used as control. All data and results obtained from evaluation of antimicrobial activities of test compounds were expressed as mean ± SEM, and were subjected to one way ANOVA followed by Dunnet test (post test) using GraphPad Prism® in stat software (Version 5.01) to determine the significance of difference between the test compounds and standard drugs. Values were considered significant when $P < 0.01$ or 0.05. The antimicrobial activity results are represented and discussed in Table no. 3.

RESULTS AND DISCUSSION

Physicochemical data of the synthesized compounds **5a-5l** including molecular formula, molecular weight, percentage yield, melting point and R_f value are shown in **Table 1.** Analytical data including IR, NMR and Mass spectral data is presented in **Table 2** whereas results of antimicrobial studies are tabulated in **Table 3**. It was found in the antimicrobial evaluation of the titled compounds that compound **5l** having P value < 0.01 was found to be the most potent antibacterial compound in this series which showed antibacterial activity comparable to that of standard drug Ciprofloxacin. Whereas compound **5c** having P value < 0.01 exhibited most potent antifungal activity when compared with standard drug Fluconazole.

Table 1: Physical Characterization Data of the Synthesized Compounds 5a-5l

Compd.	Molecular Formula	Melting Point (°C)	Molecular Weight	% Yield	R_f Value
5a	$C_{23}H_{26}N_4O_4S$	275-277	454.54	75.78	0.80
5b	$C_{22}H_{25}N_5OS$	246-248	407.54	35.0	0.58
5c	$C_{22}H_{22}N_4OS$	189-191	390.5	41.02	0.83
5d	$C_{26}H_{23}FN_4O_2S$	280-282	474.55	38.05	0.71
5e	$C_{21}H_{22}N_4O_3S$	215-217	410.49	58.67	0.63
5f	$C_{27}H_{25}FN_4O_3S$	232-234	504.58	59.64	0.71
5g	$C_{28}H_{29}N_5OS$	245-247	483.63	40.82	0.77
5h	$C_{20}H_{19}ClN_4OS$	255-257	398.91	64.26	0.62
5i	$C_{26}H_{23}ClN_4OS$	250-252	475.01	46.10	0.83
5j	$C_{20}H_{19}BrN_4OS$	188-190	443.36	74.41	0.44
5k	$C_{26}H_{23}BrN_4OS$	165-167	519.46	52.42	0.77
5l	$C_{20}H_{19}N_5O_3S$	285-287	409.46	42.71	0.72

Table 2: Analytical Data of Titled compounds 5a-5l

Compd.	IR (KBr) ν (cm^{-1})	^{1}H-NMR δ (ppm) ($CDCl_3$, 400 MHz)	MS (M^+), m/z
5a	3396.12 (NH), 2919.76 (CH-Al), 2353.14 (CH-Ar), 1483.35 (C=N), 1692.85 (C=O)	2.56-2.55 (8H, 4 CH_2), 3.59-3.35 (9H, 3 OCH_3), 8.18-7.43 (6H, Aromatic), 8.66 (1H, NH, Piperazine), 11.36 (1H, CONH), 3.24 (1H, CH, Al)	453.52
5b	3290.12 (NH), 3062.24 (CH-Al), 2354.37 (CH-Ar), 1519.25 (C=N), 1683.85 (C=O)	2.53-2.52 (8H, 4 CH_2), 2.54 (6H, 2 $N(CH_3)$, 3.35 (1H, CH, Al), 8.66-8.18 (8H, Ar), 11.36 (1H, CONH), 8.66 (1H, NH, Piperazine)	406.76
5c	3340.34 (NH), 3056.23 (CH-Al), 2335.12 (CH-Ar), 1522.25 (C=N), 1688.74 (C=O)	2.15-2.12 (8H, 4 CH_2), 3.24 (3H, 3 CH, Al), 7.56-7.48 (9H, Ar), 11.39 (1H, CONH), 8.66 (1H, NH, Piperazine)	388.94

5d	3312.51 (OH), 3273.74 (NH), 3054.74 (CH-Al), 2349.37 (CH-Ar), 1525.83 (C=N), 1688.63 (C=O), 1357.82 (C-F)	2.61-2.52 (8H, 4 CH_2), 3.64 (1H, OH), 3.36 (1H, CH, Al), 8.65-7.93 (12H, Ar), 11.33 (1H, CONH)	476.95
5e	3242.12 (OH), 3374.84 (NH), 3050.26 (CH-Al), 2297.75 (CH-Ar), 1528.83 (C=N), 1704.25 (C=O)	2.54-2.48 (8H,4 CH_2), 3.52 (1H, OH), 3.73 (3H, OCH_3), 3.37 (1H, CH, Al), 7.65-7.48 (7H, Ar), 11.32 (1H, CONH), 8.66 (1H, NH, Piperazine)	409.65
5f	3312.12 (OH), 3271.94 (NH), 2917.32 (CH-Al), 2349.39 (CH- Ar), 1525.26 (C=N), 1690.94 (C=O), 1053.27 (C-F)	2.54-2.53 (8H,4 CH_2), 2.65 (3H, OCH_3) 3.67 (1H, OH), 3.66 (1H, CH, Al), 8.85-7.52 (11H, Ar), 11.35 (1H, CONH)	506.53
5g	3272.21 (N-H), 2917.83 (CH-Al), 2354.75 (CH-Ar), 1525.52 (C=N), 1684.10 (C=O)	2.57-2.54 (8H, 4 CH_2), 2.54-2.53 (6H, N-CH_3), 3.34 (1H, CH, Al), 8.85-7.52 (13H, Ar), 11.40 (1H, CONH)	482.59
5h	3272.23 (NH), 2891.74 (CH-Al), 2312.94 (CH-Ar), 1525.75 (C=N), 1692.63 (C=O), 750.72 (C-Cl)	2.53-2.52 (8H, 4 CH_2), 3.66 (1H, CH, Al), 8.85-7.52 (8H, Ar), 11.41 (1H, CONH), 8.66 (1H, NH, Piperazine)	399.71
5i	3272.23 (NH), 2918.84 (CH-Al),2349.45 (CH-Ar), 1526.83 (C=N), 1680.32 (C=O), 751.28 (C-Cl)	2.54-2.53 (8H, 4 CH_2), 3.42 (1H, CH, Al), 8.88-7.51 (13H, Ar), 11.36 (1H, CONH)	477.83
5j	3272.23 (NH), 2917.54 (CH-Al), 2354.64 (CH-Ar), 1525.28 (C=N), 1680 (C=O), 663. 38 (C-Br)	2.57-2.53 (8H, 4 CH_2), 3.41 (1H, CH, Al), 8.85-7.52 (8H, Ar), 11.41 (1H, CONH), 8.66 (1H, NH, Piperazine)	444.58
5k	3276.23 (NH), 2956.93 (CH-Al), 2354.35 (CH-Ar), 1526.13 (C=N), 1692.74 (C=O), 723.42 (C-Br)	2.54-2.53 (8H, CH_2), 3.66 (1H, CH, Al), 8.85-7.52 (13H, Ar), 11.35 (1H, CONH)	521.64
5l	3272.22 (N-H), 2952.96 (CH-Al), 2349.61 (CH-Ar), 1526.53 (C=N), 1683 (C=O), 1356.23 (N=O, Nitro)	2.53-2.52 (8H, CH_2), 3.33 (1H, CH, Al), 8.64-8.15 (8H, Ar), 11.41 (1H, CONH), 8.66 (1H, NH, Piperazine)	408.26

Table 3: Antimicrobial Activity of Test Compounds 5a-5l

Comp. Code	R_1	R_2	Zone of Inhibition Values (mm) ± SEM		
			***Staphylococcus aureus* (ATCC 6538)**	***Escherichia coli* (ATCC 10798)**	***Candida albicans* (ATCC 10231)**
5a	3,4,5-OCH_3	H	12.46 ± 0.054	11.86 ± 0.063	23.45 ± 0.053
5b	4-$N(CH_3)_2$	H	19.48 ± 0.053	17.56 ± 0.060	21.40 ± 0.064
5c	CH_3CH=CH-C_6H_4	H	16.90 ± 0.043	15.25 ± 0.046	24.76 ± 0.053

5d	2-OH	4-F - C_6H_4	22.46 ± 0.025	14.81 ± 0.011	18.12 ± 0.032
5e	2-OH, 4-OCH3	H	20.74 ± 0.064	18.79 ± 0.053	20.18 ± 0.064
5f	2-OH, 4-OCH_3	4-F - C_6H_4	17.88 ± 0.011	16.31 ± 0.041	19.74 ± 0.012
5g	4-$N(CH_3)_2$	C_6H_5	18.30 ± 0.063	18.46 ± 0.053	22.96 ± 0.035
5h	4-Cl	H	21.46 ± 0.019	21.81 ± 0.034	15.96 ± 0.068
5i	4-Cl	C_6H_5	26.81 ± 0.063	24.10 ± 0.053	17.35 ± 0.063
5j	4-Br	H	23.18 ± 0.023	21.42 ± 0.046	14.18 ± 0.012
5k	4-Br	C_6H_5	22.77 ± 0.055	20.51 ± 0.060	16.10 ± 0.067
5l	4-NO_2	H	27.68 ± 0.033	25.58 ± 0.060	10.45 ± 0.042
Control	NA	NA	-	-	-
Ciprofloxacin	NA	NA	29.52 ± 0.057	27.26 ± 0.068	-
Fluconazole	NA	NA	-	-	25.36 ± 0.056

CONCLUSION

A novel series of benzothiophene incorporated piperazine derivatives **5a-5l** were synthesised and evaluated for their antimicrobial activities. All of the synthesized compounds showed significant antimicrobial activities but one compound **5l** with $p < 0.01$ having electron withdrawing nitro group at para position of phenyl ring exhibited most potent antibacterial activity when compared with standard drug Ciprofloxacin. Compound **5c** with $p < 0.01$ having 3-phenylprop-2-en-1-imine group displayed the most antifungal activity when compared with standard drug Fluconazole. It was also observed that the remaining compounds showed mild to moderate antimicrobial activities when compared with their respective standard drugs. The encouraging antimicrobial actitity results of benzothiophene incorporated piperazine derivatives may be utilized significantly by medicinal chemists for the drug design and development of novel antimicrobial agents.

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